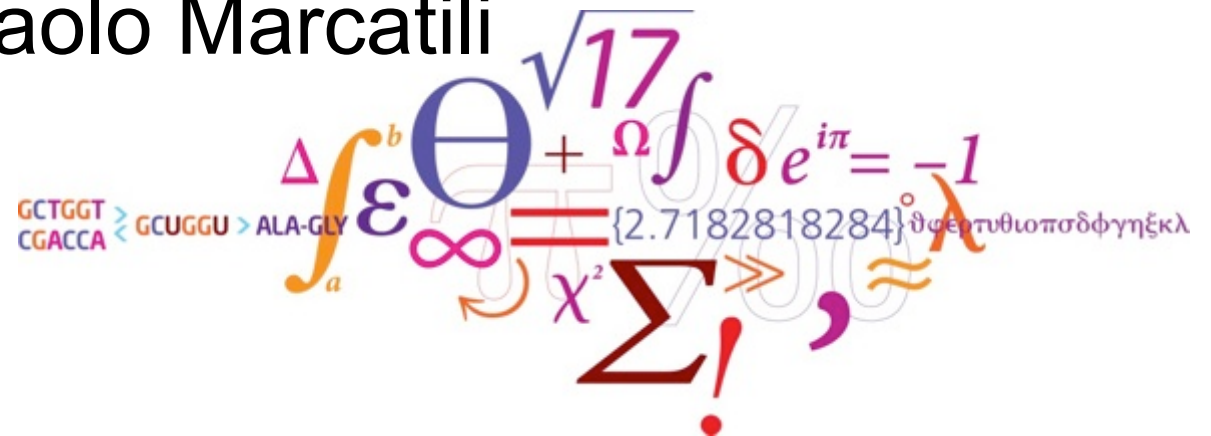


Digital Dermatitis

what immunological bioinformatics can do
for cows

Paolo Marcatili



Digital Dermatitis

Recent disease (1975, Italy)

Milk yield loss (.5 - .75 l/day)

Economic loss ~ 350 dkk per cow/year

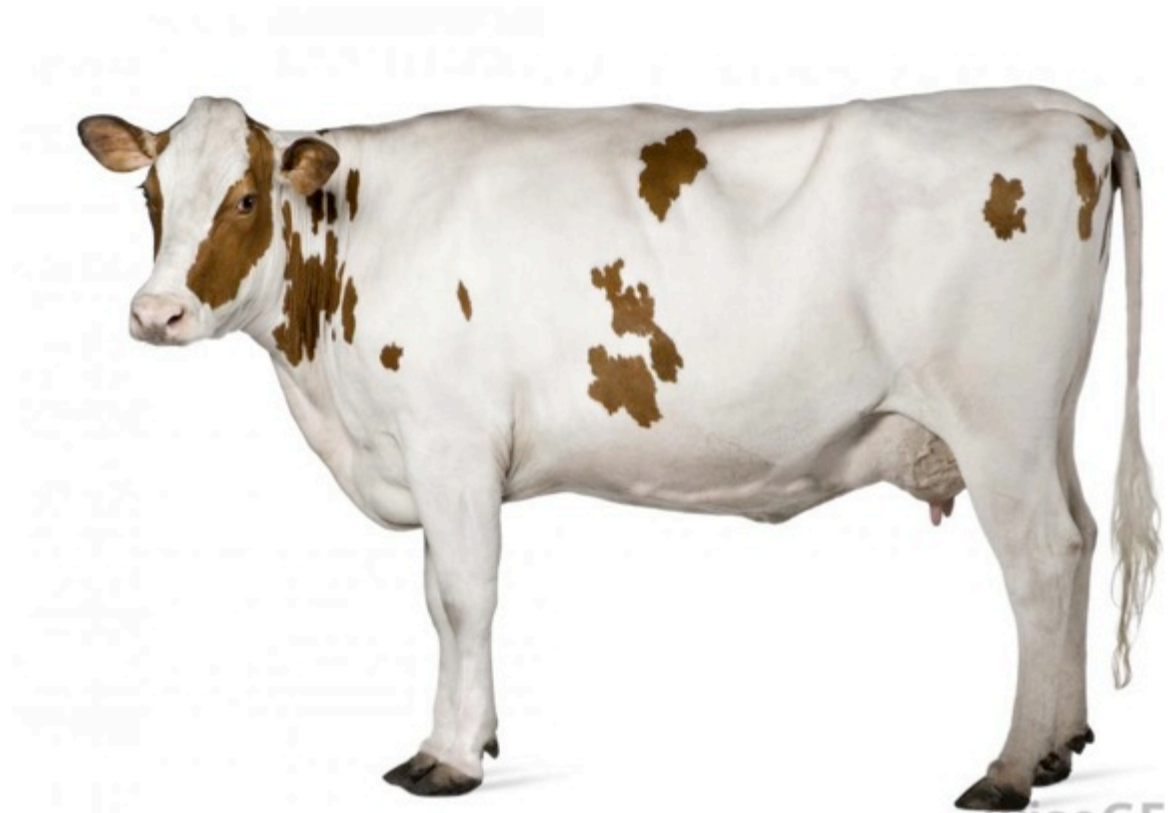
Bacterial interplay (Treponemas)

40% of cows infected



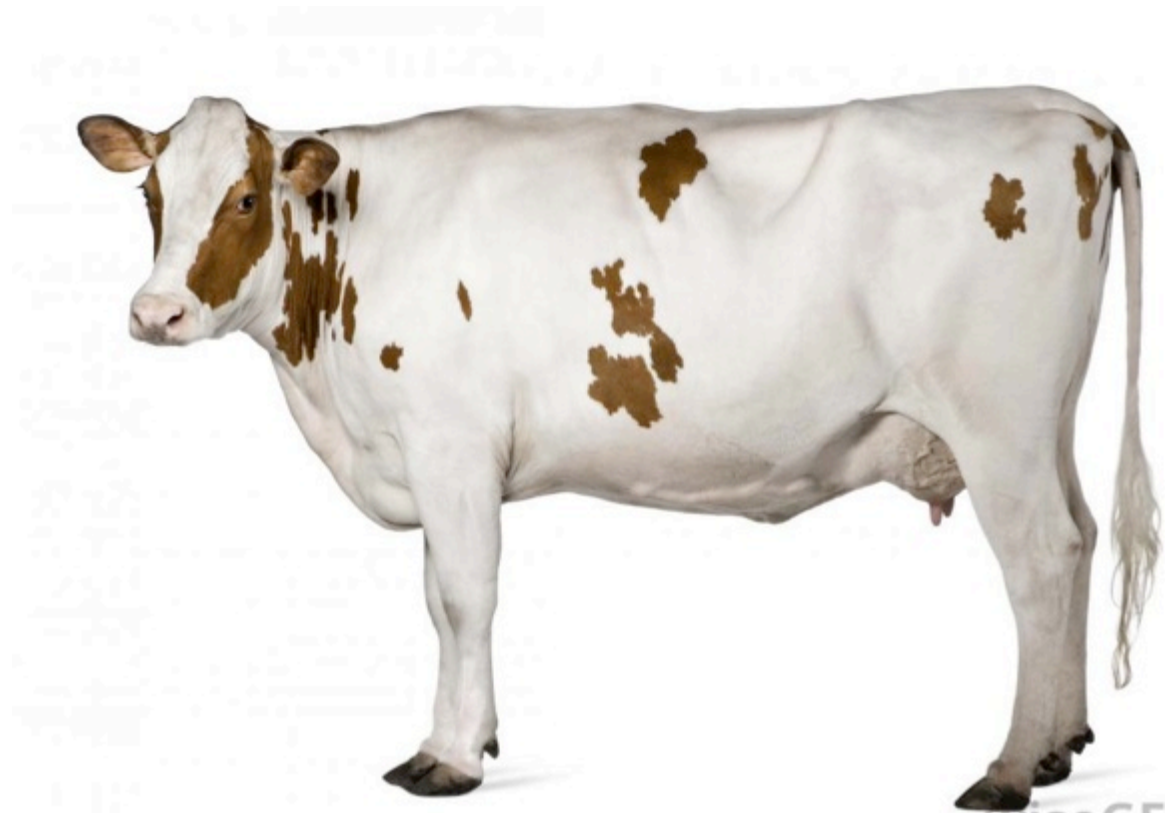
Digital Dermatitis

Discover Antigens
Vaccine



Digital Dermatitis

Discover Antigens
~~Vaccine~~
Markers



Digital Dermatitis

Discover Antigens
~~Vaccine~~
Markers



Digital Dermatitis

Open lesions near the hoofs



Digital Dermatitis

Open lesions near the hoofs

Opportunistic infections



Digital Dermatitis

Open lesions near the hoofs

Opportunistic infections

Foot Baths with antibiotics



Question:

Diverse Multimicrobial Environment +
non-specific, long term antibiotic treatment =

?

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non-specific, long term antibiotic treatment =

Antibiotic resistance!

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Human Pathogen

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Antibiotic resistance!

Human Pathogen

that causes **periodontitis**

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Diverse Multimicrobial Environment +

non-specific, long term antibiotic treatment =

Antibiotic resistance!

Human Pathogen

that causes periodontitis

similar to the **syphilis pathogen**
(horizontal gene transfer)

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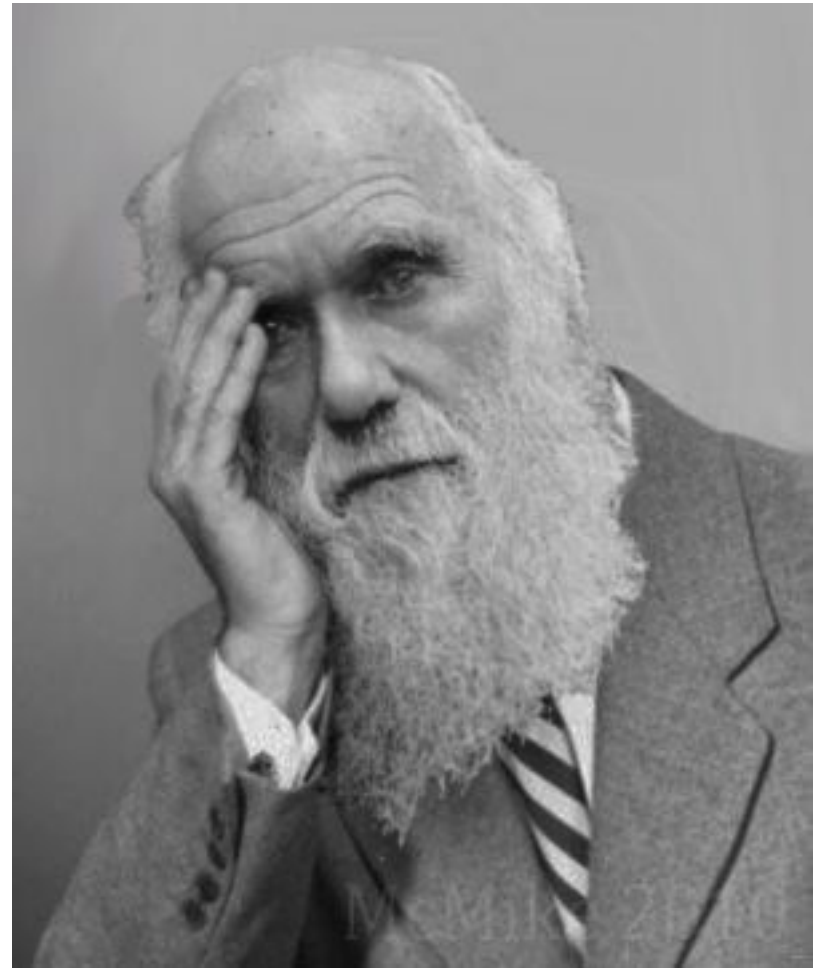
Diverse Multimicrobial Environment +
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Antibiotic resistance!

Human Pathogen

that causes periodontitis

similar to the **syphilis pathogen**
(horizontal gene transfer)



Guns, Germs, and Steel

Evolution of epidemic infectious diseases

The main killers of humans since the advent of agriculture have been **acute, highly infectious, epidemic diseases** that are confined to humans and that either kill the victim quickly or, if the victim recovers, immunize him/her for life.

Nature **418**, 700-707 (8 August 2002) | doi:10.1038/nature01019

review article

Evolution, consequences and future of plant and animal
domestication

Jared Diamond

Guns, Germs, and Steel

Evolution of epidemic infectious diseases

Such diseases could not have existed before the origins of agriculture, because they can sustain themselves only in large dense populations that did not exist before agriculture, hence they are often termed '**crowd diseases**'.

Nature **418**, 700-707 (8 August 2002) | doi:10.1038/nature01019

review article

Evolution, consequences and future of plant and animal
domestication

Jared Diamond

Guns, Germs, and Steel

Evolution of epidemic infectious diseases

The mystery of the origins of many of these diseases has been solved by molecular biological studies of recent decades, demonstrating that they evolved from similar epidemic diseases of our **herd domestic animals** with which we began to come into close contact **10,000 years ago**.

Nature **418**, 700-707 (8 August 2002) | doi:10.1038/nature01019

review article

Evolution, consequences and future of plant and animal domestication

Jared Diamond

Guns, Germs, and Steel

Evolution of epidemic infectious diseases

Thus, the evolution of these diseases depended on two separate roles of domestication: in creating **much denser human populations**, and in permitting much more frequent **transmission of animal diseases** from our domesticates than from hunted wild animals.

Nature **418**, 700-707 (8 August 2002) | doi:10.1038/nature01019

review article

Evolution, consequences and future of plant and animal domestication

Jared Diamond

Guns, Germs, and Steel

Evolution of epidemic infectious diseases

For instance, measles and **tuberculosis** arose from diseases of **cattle**, **influenza** from a disease **of pigs and ducks**.

An outstanding mystery remains the origins of **smallpox**:
did it reach us from **camels or from cattle**?

Nature **418**, 700-707 (8 August 2002) | doi:10.1038/nature01019

review article

Evolution, consequences and future of plant and animal
domestication

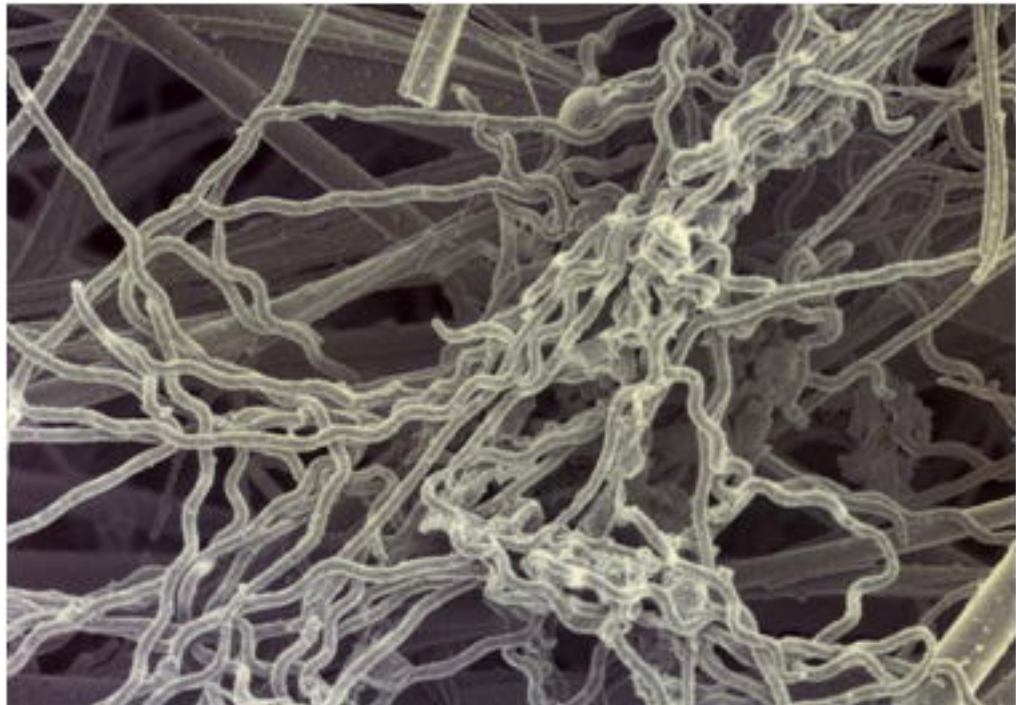
Jared Diamond

Vaccine!



The suspects

Treponema denticola
Treponema pedis
Treponema phagedenis
...



Question I

What is the etiologic agent

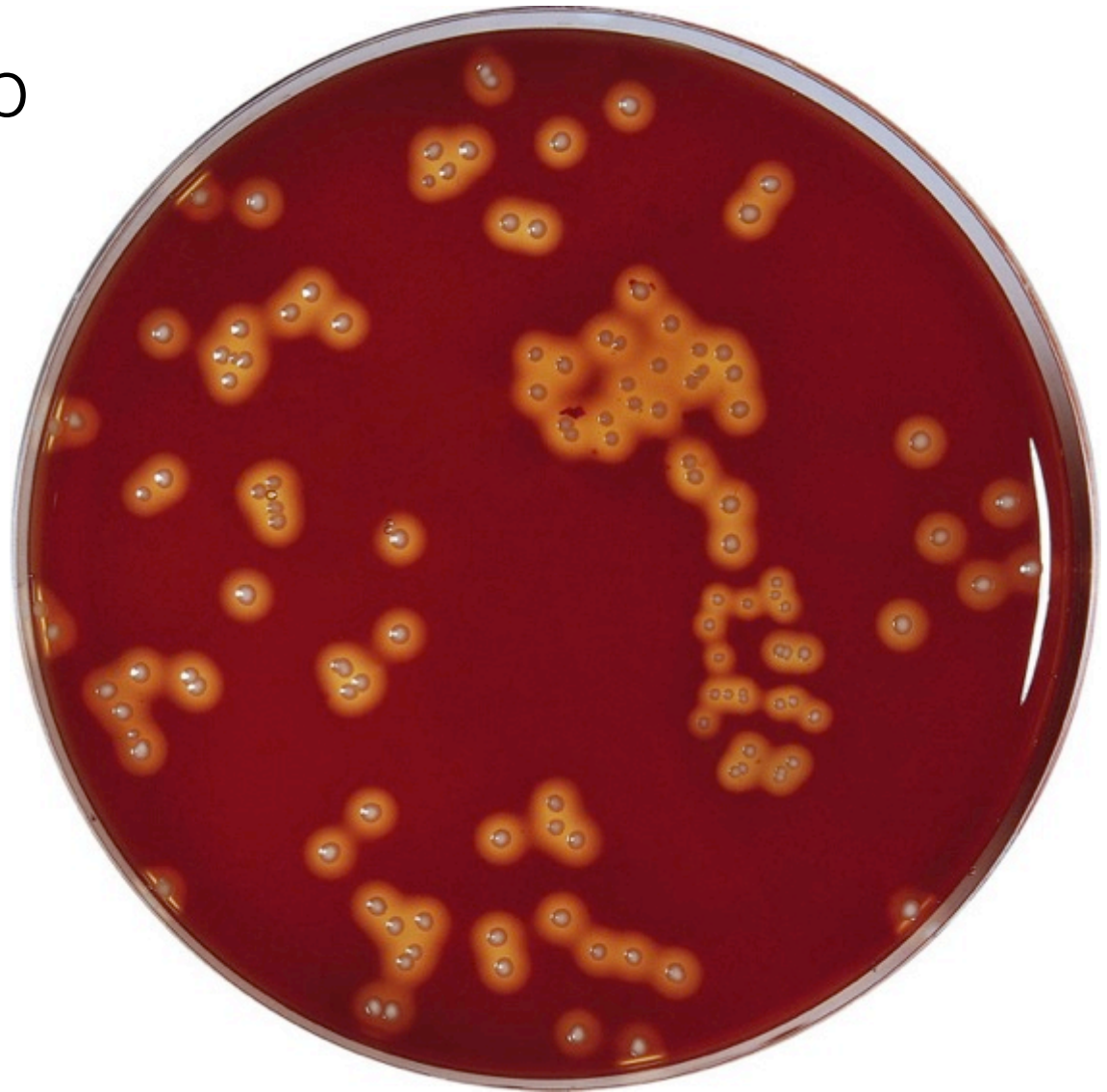
What are the proteins expressed and in which stage

Which are the epitopes

Which epitopes are effective

Experimental setup

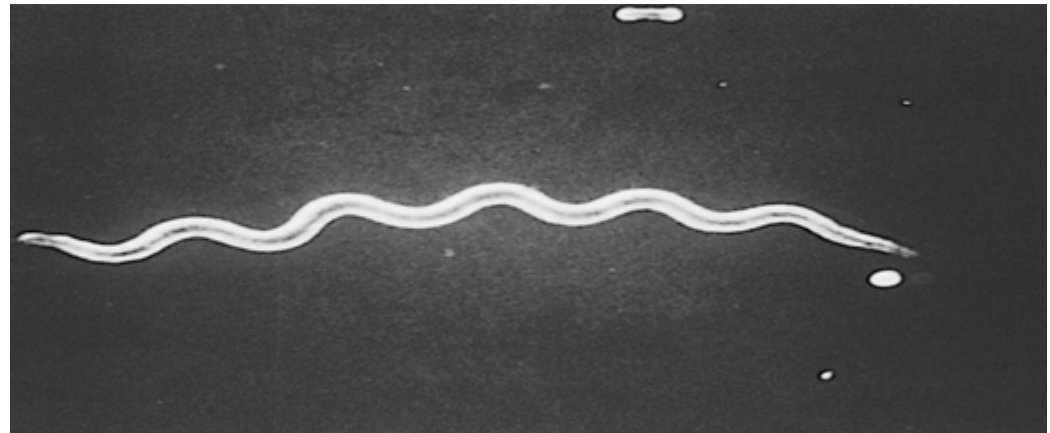
In vitro culture



Experimental setup

~~*In vitro*~~ culture

Genomics

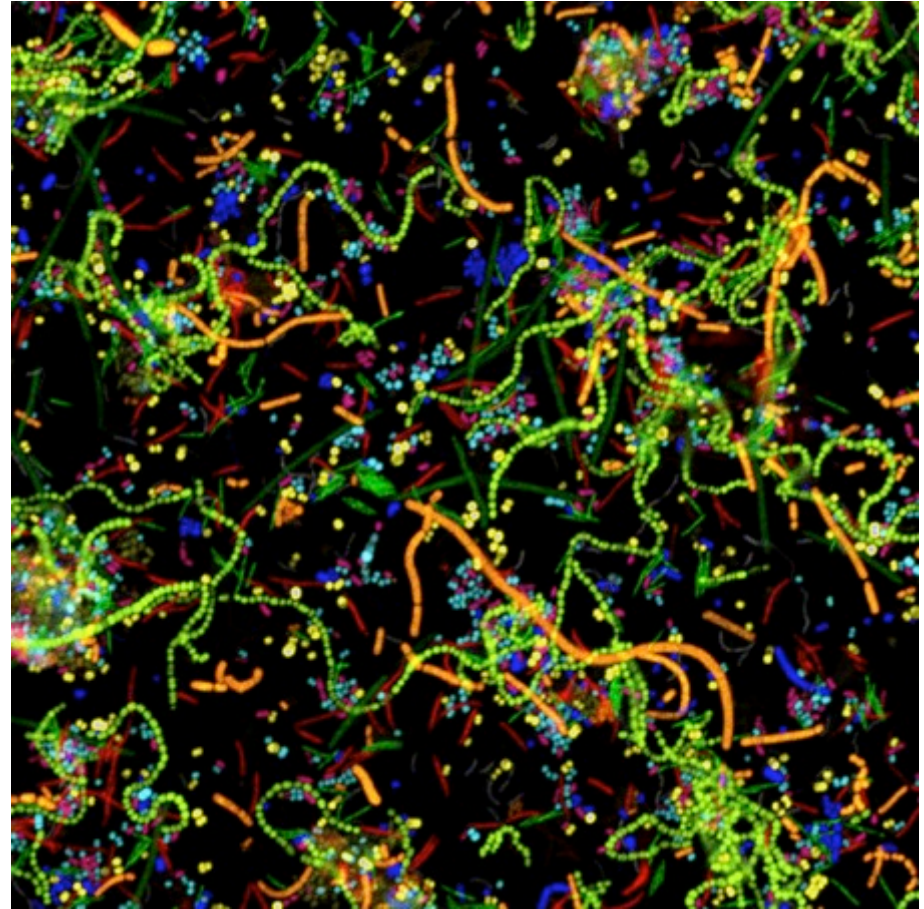


Experimental setup

~~*In vitro*~~ culture

~~Genomics~~

Metagenomics



Experimental setup

In vitro culture

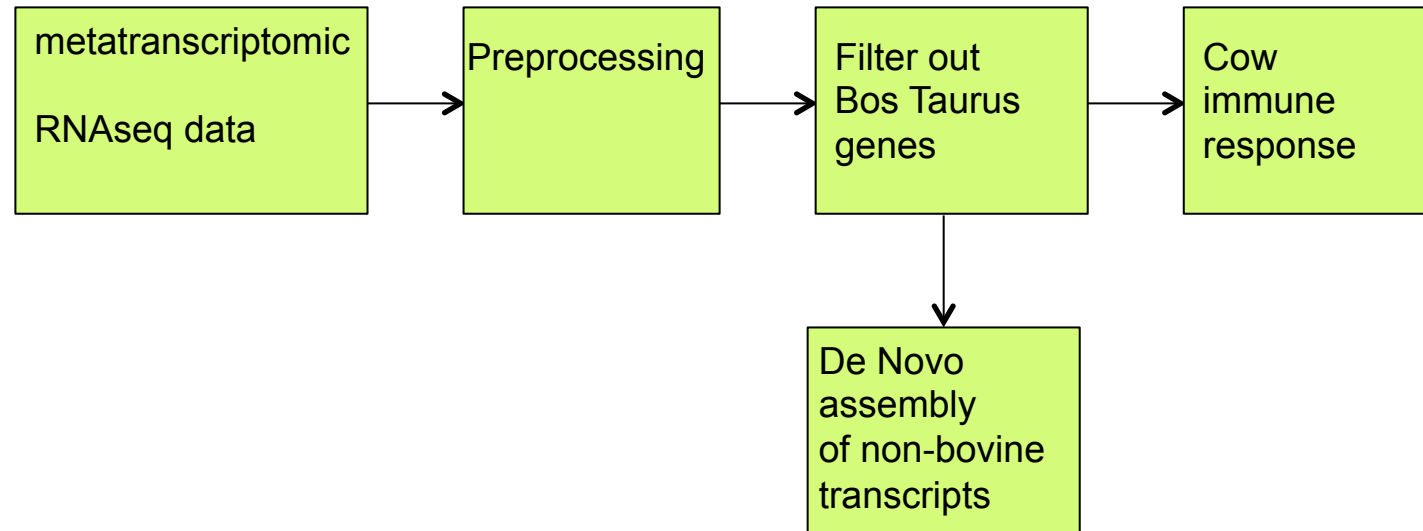
Genomics

Metagenomics

Population metagenomics



Integrated vaccine discovery pipeline



RNAseq data

A snapshot of RNA content of the sample

Illumina, 100 bp reads, strand specific

32 samples

1 healthy cow

28 sick cows (biopsies and live samples)

on average, 45M reads per sample

reads= short stretches of nucleotides which are amplified and detected

Each read comes with its own base-specific quality control



Preprocessing NGS data

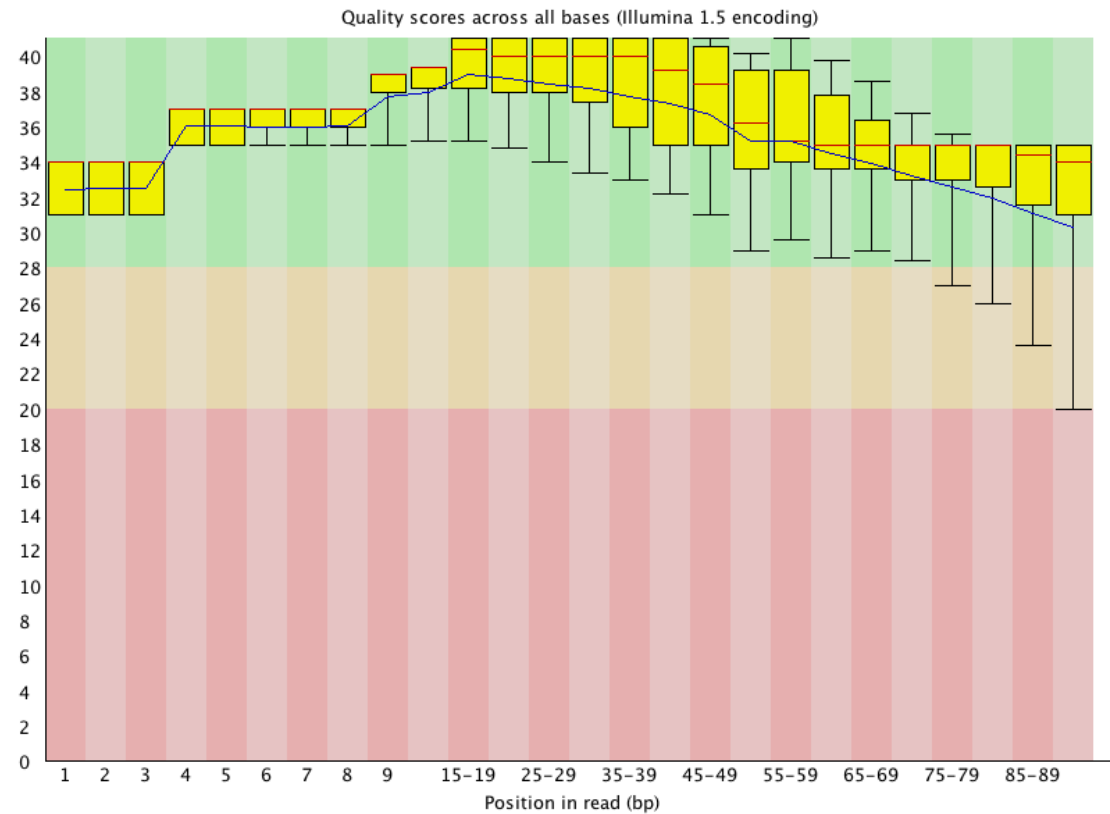
Low quality reads / bases

Poor quality at 3'

Sequencing Artifacts

Indels

null base calls



Filtering

We filter out Cow transcripts and Ribosomal RNA

!! more than 90% of (untreated) RNA content is ribosomal!!

This process filters out more than 95% of our reads

De Novo assembly

Remaining reads are assembled **without a reference genome**

- Noisy,
- sensible to parameters and seq. errors
- requires lot of data
- lot of computational resources
- hard to annotate

Final amount: 78.000 transcripts, 55% read remapping

De Novo assembly

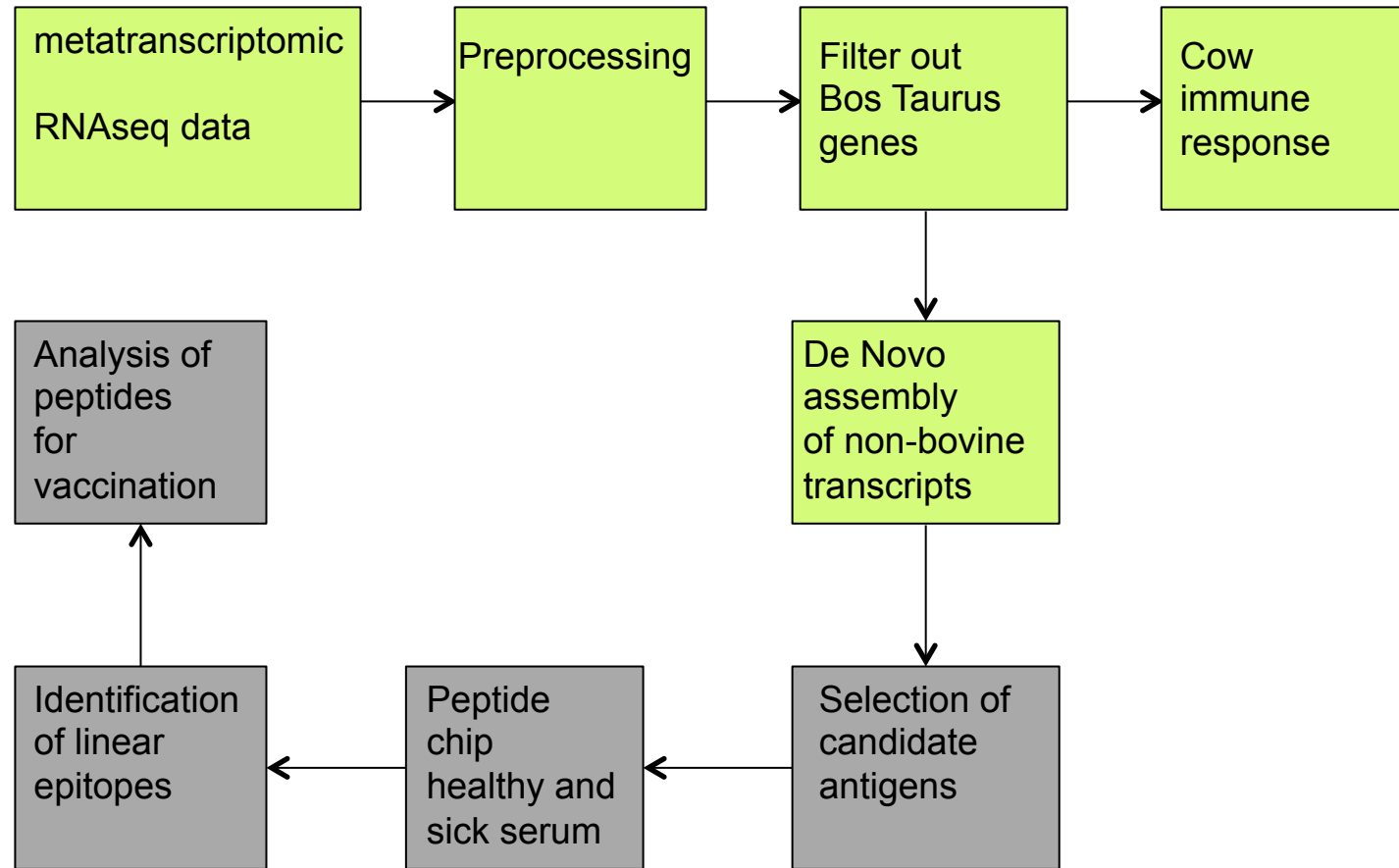
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- sensible to parameters and seq. errors
- requires lot of data
- lot of computational resources
- hard to annotate

Final amount: 78.000 transcripts, 55% read remapping

Data does not saturate the transcripts

Integrated vaccine discovery pipeline



Putative antigens

80.000 non bovine transcripts assembled

Contaminations (Man, Mouse, ...)

Low abundance (Transcripts observed in < 10 samples)

Non-antigenic proteins (Signal peptide, membrane, Pfam domains)

Putative antigens

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- blast on annotated dbs to infer taxonomy / function

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Non-antigenic proteins (Signal peptide, membrane, Pfam domains)

- Prediction tools (NN, HMMs, ...)

Putative antigens

80.000 non bovine transcripts assembled

5.000 candidate proteins

From proteins to peptides

Only ~10% of epitopes are linear

but

~50% have a linear core

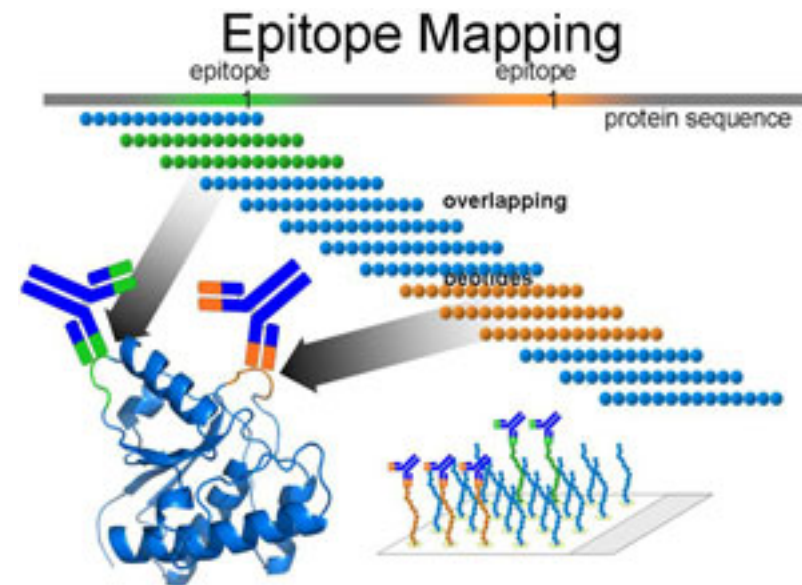
Screening using peptide arrays

500.000 to 5M peptides on one chip

Modifications are possible

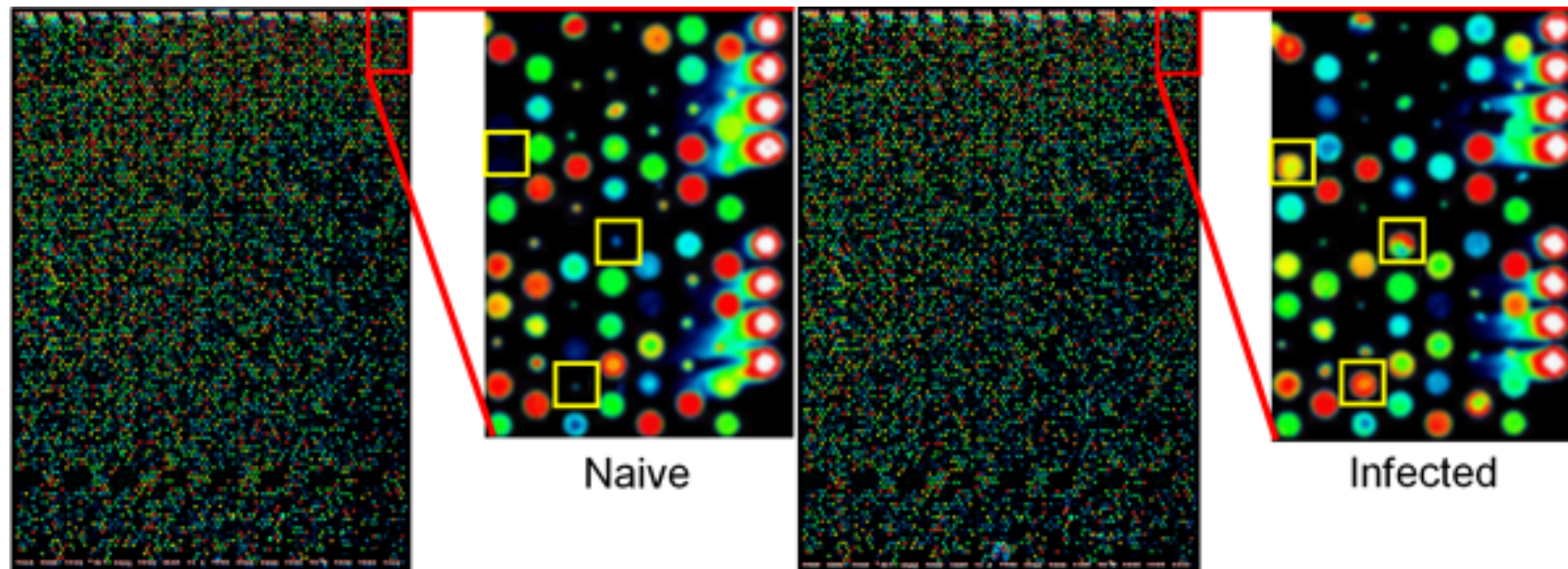
Unexpensive

Fast



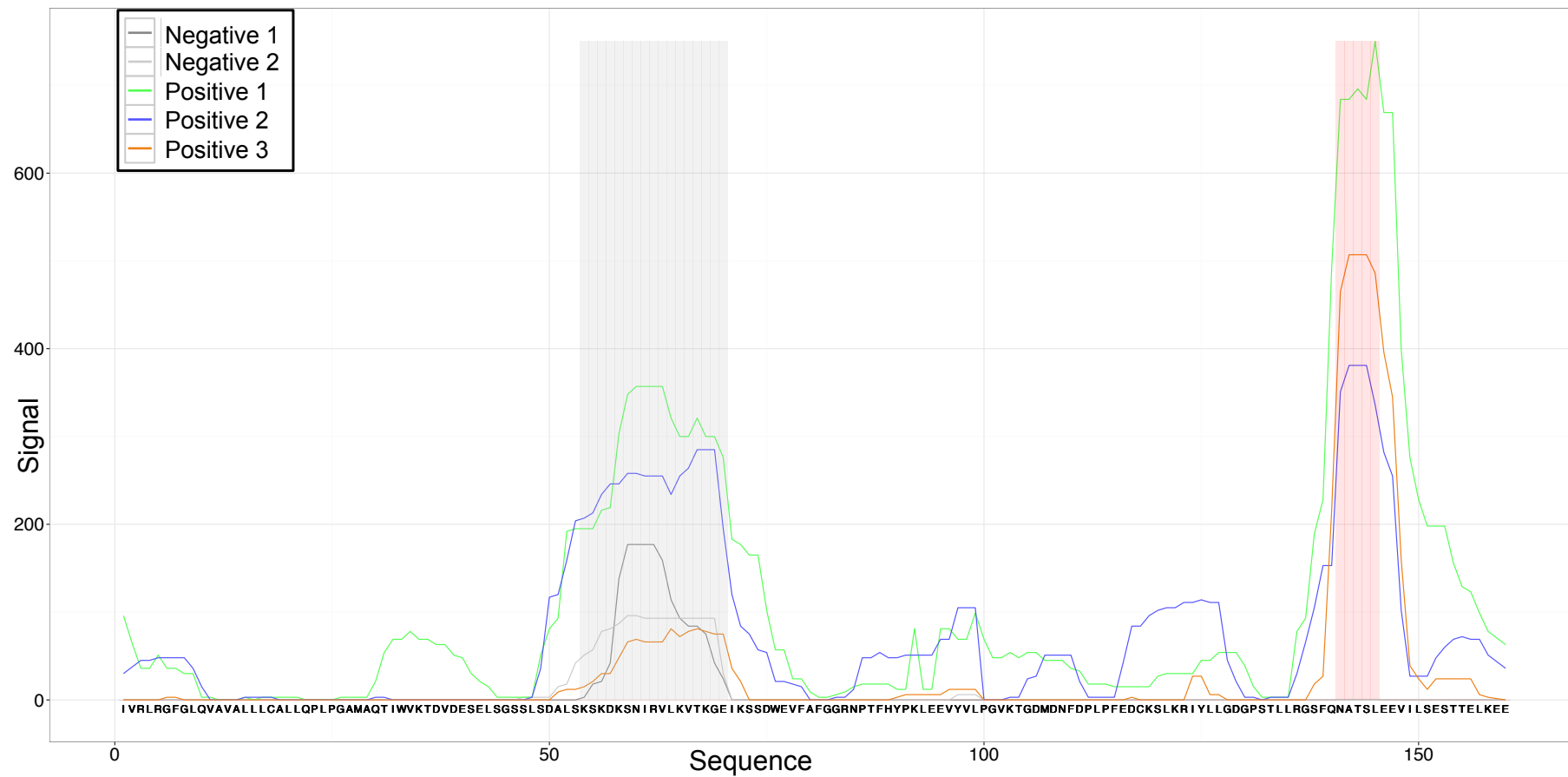
From proteins to peptides

Which peptides react with the serum from sick cows **ONLY**

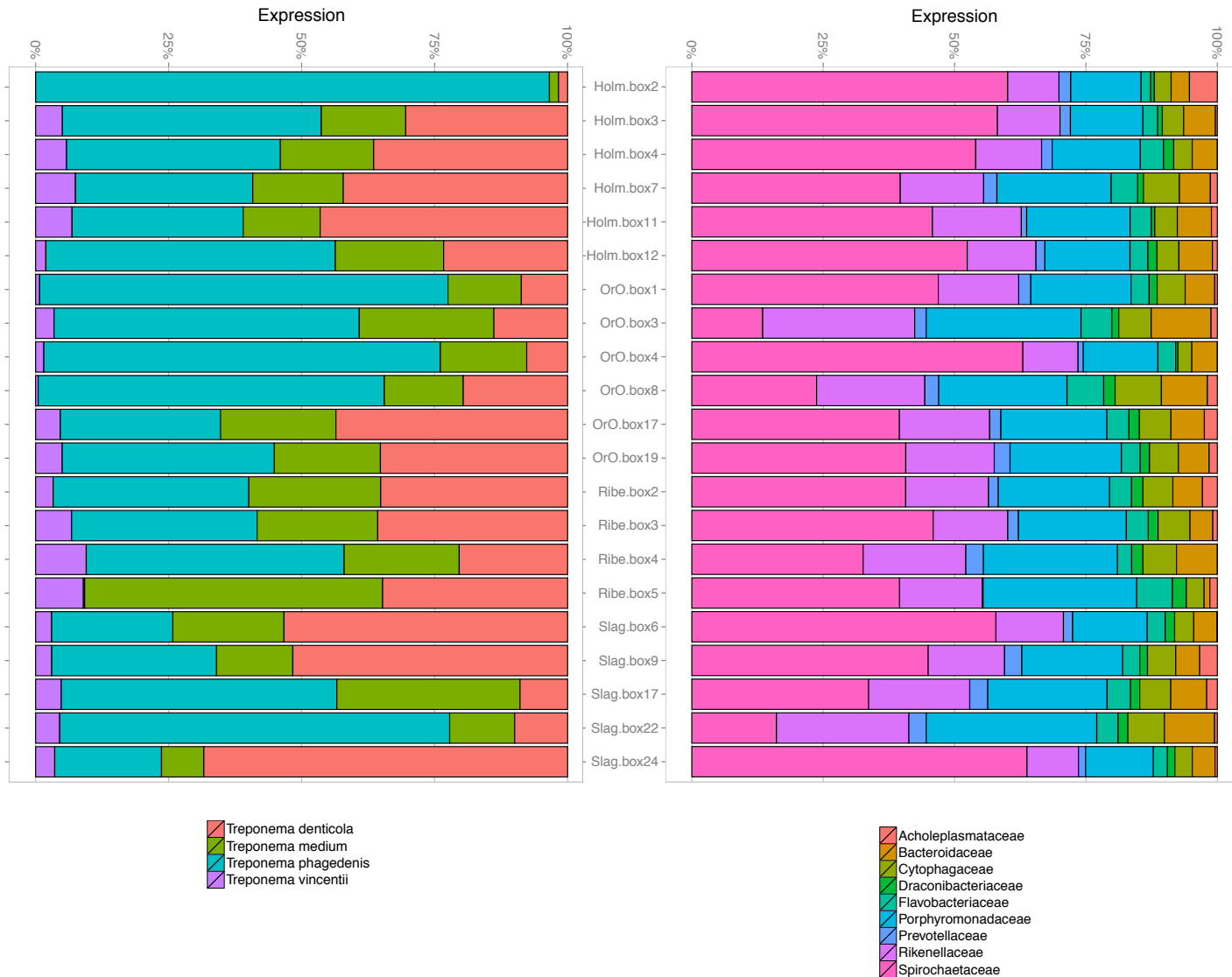


From proteins to peptides

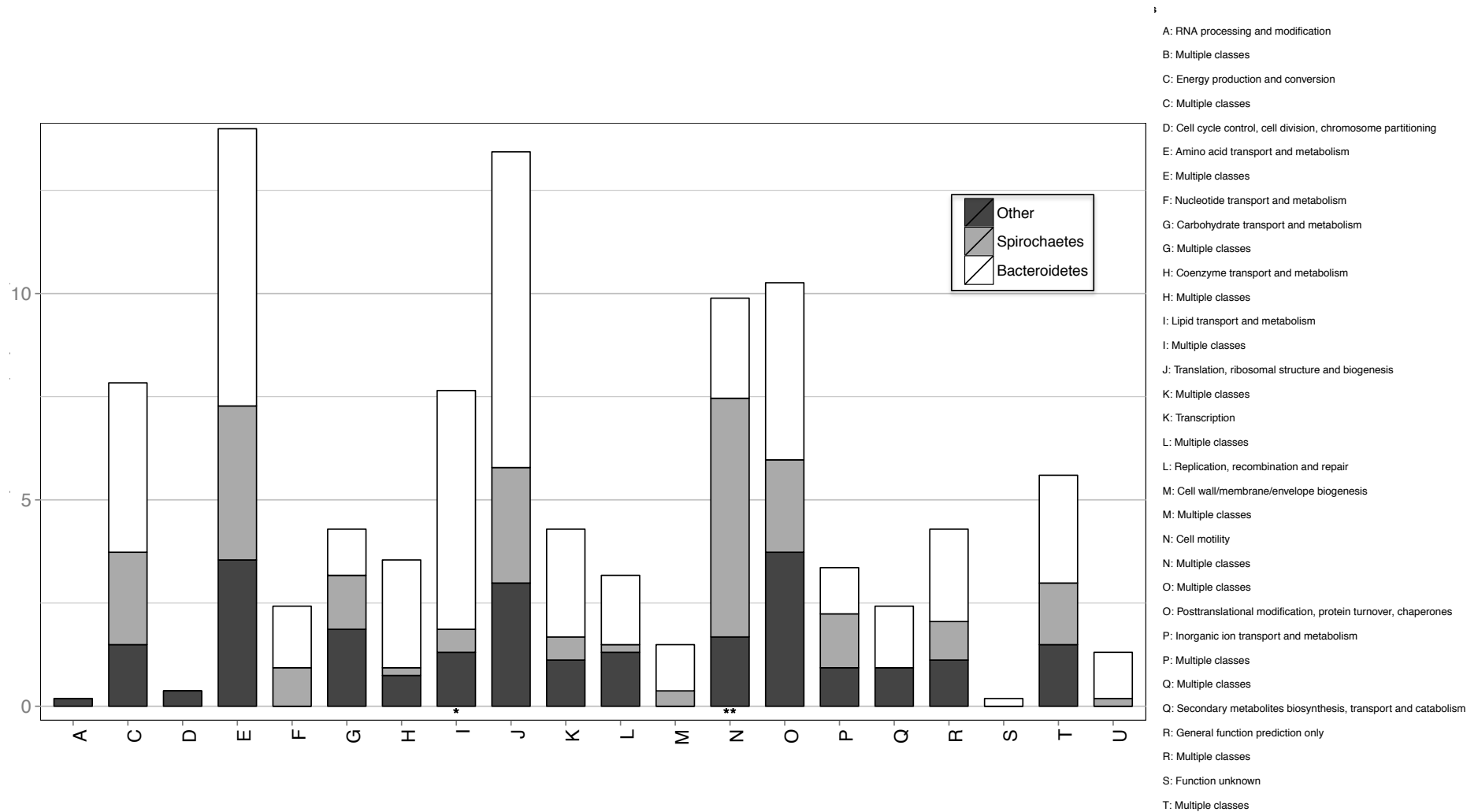
Which peptides react with the serum from sick cows **ONLY**



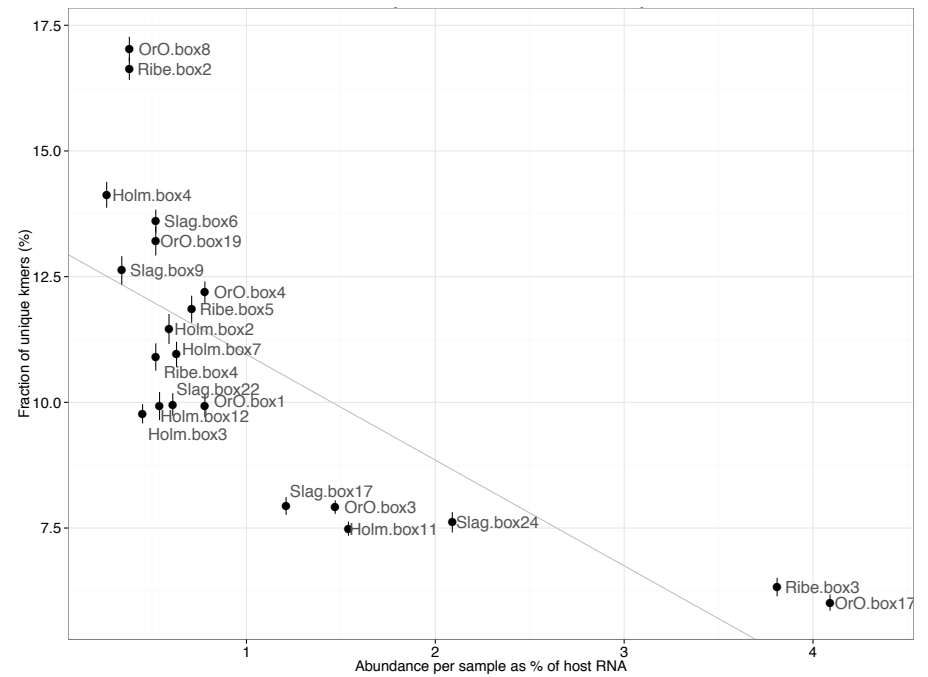
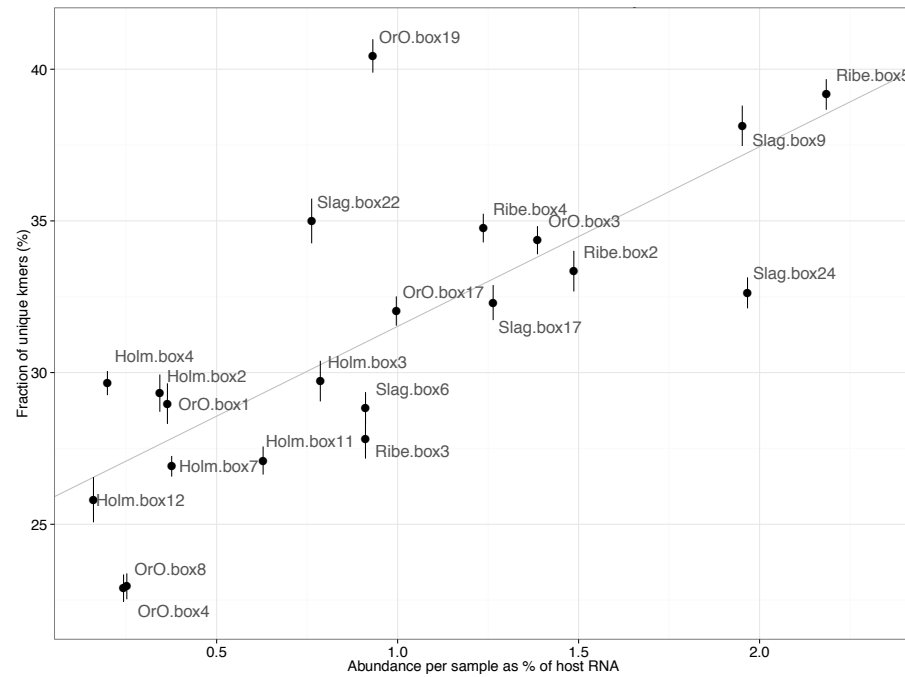
Taxonomy of a polyclonal response



Functional analysis of antigens



Pathogen Vs Host



Take Home messages

- Most 'low-hanging' vaccines have been picked
- New tools (NGS, peptide chips, protein engineering)
- Neglected and domestic animal diseases
- Antibiotic overuse in animals is a major threat

We will see most of these things in detail in the next days!